Treatment-induced senescence in metastatic colorectal adenocarcinomas: implications for single-cell biology and therapeutic intervention

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Introduction

In cancer, the deleterious role of senescence is now well recognized and is largely mediated by the senescence-associated secretory phenotype (SASP), characterized by the local production of pro-inflammatory cytokines, immunosuppressive soluble factors and metalloproteinases. Many chemotherapy and radiotherapy regimens induce senescence in tumor cells and promote a harmful microenvironment that favors tumor growth, invasion, neoangiogenesis, treatment resistance, relapse and poor prognosis, particularly in advanced disease.

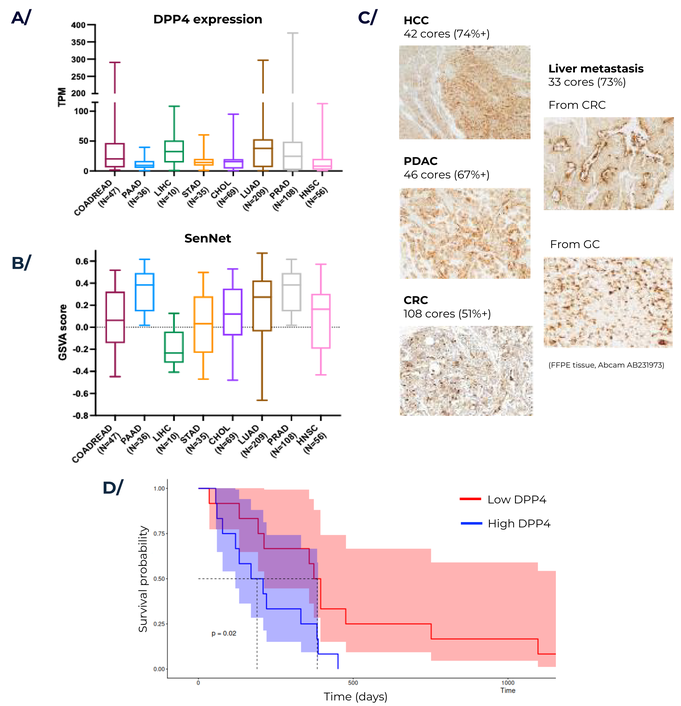
Among senescence biomarkers, dipeptidyl peptidase 4 (DPP4/CD26), a membrane glycoprotein involved in chemotactic and inflammatory responses, is frequently constitutively expressed and/or overexpressed in primary tumors and metastases, including metastatic colorectal adenocarcinoma (mCRC). *See poster #1692/21 – QR code in the discussion.*

To investigate the pathophysiology of SOC-induced senescence, we analyzed a large series of mCRC, other digestive cancers and hepatic carcinomas. DPP4 expression was assessed by immunohistochemistry before and after SOC and correlated with clinical annotations and other senescence features in primary tumors and liver metastases (n=100 samples). In parallel, a comprehensive bioinformatic analysis was performed on a large pan-tumor bulk RNA-sequencing dataset (n=980 samples), evaluating a broad panel of candidate genes and senescence-related signatures, including DPP4, using MSigDB, CellMarker2.0, PanglaoDB and SenNet. The same analyses were conducted in multiple scRNA-seq mCRC cohorts (n=105 patients), including treatment-naïve primary tumors or liver metastases (n=73) and post-SOC biopsies (n=54).

To our knowledge, this is the first study to demonstrate, at both the protein and single-cell RNA levels, the deleterious impact of senescence across a large series of patients with mCRC during cancer progression and treatment.

These findings may have important implications for the development of innovative therapies targeting senescence biomarkers and related signaling pathways to eliminate cancer cells in refractory tumors.

Fig. 1 DPP4 is Expressed Across Multiple Solid Tumor Indications



A/ DPP4 expression in Transcript per million (TPM) in different type of cancers (TCGA database).
B/ Distribution of GSVA senescence signature scores across cancer types (SenNet database4) (COADREAD: Colorectal Adenocarcinoma, PAAD: Pancreatic Adenocarcinoma, LIHC: Liver Hepatocellular Carcinoma, STAD: Stomach Adenocarcinoma, CHOL: Cholangiocarcinoma , LUAD/ Lung Adenocarcinoma, PRAD: Prostate Adenocarcinoma, HNSC: Head-Neck Squamous Cell Carcinoma).
C/ DPP4 expression analysis by immunohistochemistry on Tissue MicroArray (TMA) from pancreatic, colon, liver carcinoma and liver metastasis.
D/ Survival probability of patients with colon adenocarcinoma in function of DPP4 expression (low DPP4 in red and high DPP4 in blue).

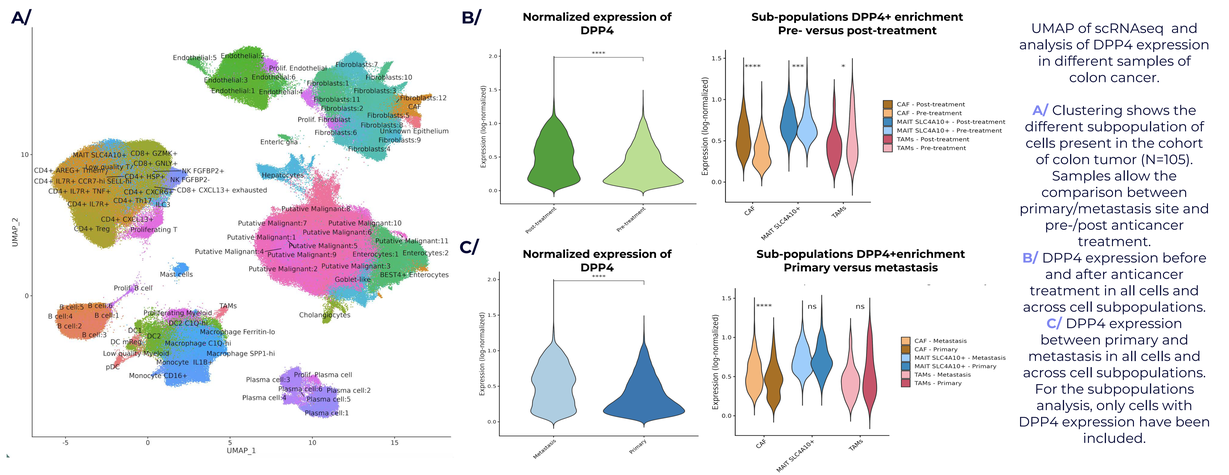
Materials & Methods

Bioinformatics pipelines were designed to address correlations of candidate genes and molecular signatures to cellular heterogenicity, context, biology and clinical outcomes performed on two sets of databases:

- pan-tumor bulk RNASeq database (n=980 samples from metastases of various localization across TCGA indications);
- scRNASeq mCRC database (n=127 samples derived from pre-/post SoC primary tumor (n=67/14) and liver metastatic samples (n=6/40).

All tumor samples were collected at Gustave Roussy with patients signed informed consent enrolled during the course of the precision medicine programs Moscato [NCT01566019] and Match-R [NCT02517892]. The present study was approved by the internal scientific review board of Gustave Roussy [n°2025-692].

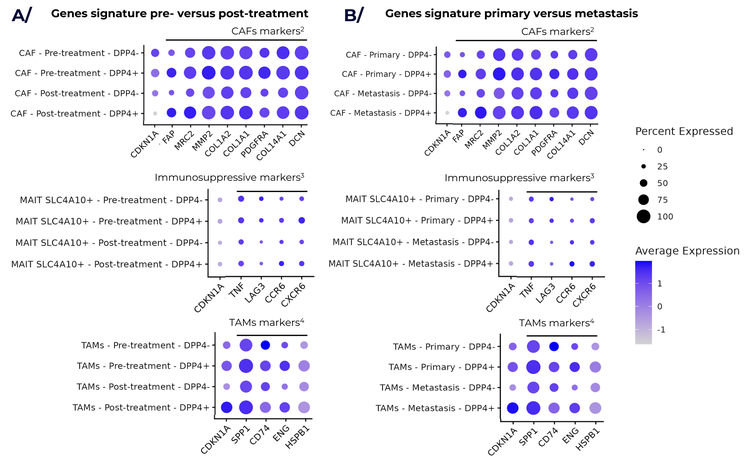
Fig. 2 ScRNAseq shows up-regulation of DPP4 in colon adenocarcinoma



UMAP of scRNAseq and analysis of DPP4 expression in different samples of colon cancer.

A/ Clustering shows the different subpopulation of cells present in the cohort of colon tumor (N=105). Samples allow the comparison between primary/metastasis site and pre-/post anticancer treatment.
B/ DPP4 expression before and after anticancer treatment in all cells and across cell subpopulations.
C/ DPP4 expression between primary and metastasis in all cells and across cell subpopulations. For the subpopulations analysis, only cells with DPP4 expression have been included.

Fig. 3 Biological signature of cell subpopulations depending on DPP4 expression



The different genes are specific markers of each populations described in the literature.

References

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Discussion

- DPP4 is expressed in different solid tumor indications and a biomarker associated with conventional senescent signature.
- In CRC, DPP4 high expression is significantly correlated with poor survival and may become useful to identify the most refractory patients with unmet medical need using targeted innovative molecules.
- ScRNAseq analysis of CRC highlights an enrichment of DPP4-postive cells after SoC treatments and at an advanced metastatic stage.
- In addition, our study evidences in the CRC samples analyzed the accumulation of discrete cell subpopulations associated to cancer (CAF, TAM and immune cells) enriched in the DPP4-positive cell fraction in metastases and post-SoC treatment.
- Overall, our results strongly support the rational for direct tumor targeting and stromal depletion of immuno-suppressive CAFs and TAMs using an anti-DPP4 ADC.

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